Effect of solute and matric potential on growth rate of fungal species isolated from cheese

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A B S T R A C T

The effect of water potential (Ψ_w) on the growth of 15 fungal species isolated from cheeses was analysed. The species, identified mainly by analysis of DNA sequences, belonged to genera *Penicillium*, *Geotrichum*, *Mucor*, *Aspergillus*, *Microascus* and *Talaromyces*. Particularly, the effect of matric potential (Ψ_m), and ionic (NaCl) and non-ionic (glycerol) solute potentials (Ψ_s) on growth rate was studied. The response of strains was highly dependent on the type of Ψ_w. For Ψ_s, clear profiles for optimal, permissive and marginal conditions for growth were obtained, and differences in growth rate were achieved comparing NaCl and glycerol for most of the species. Conversely, a sustained growth was obtained for Ψ_m in all the strains, with the exception of *Aspergillus pseudoglaucus*, whose growth increased proportionally to the level of water stress. Our results might help to understand the impact of environmental factors on the ecophysiology and dynamics of fungal populations associated to cheeses.

1. Introduction

Moulds play a determinant role on quality of cheeses. The presence of some species might be necessary for the development of a particular appearance, and rheological and sensory characteristics. Such is the case of blue veined cheeses (e.g., Roquefort) and surface mould-ripened cheeses (e.g., Camembert), where *Penicillium roqueforti* and *Penicillium camemberti*, respectively, contribute to the formation of texture and flavour through the action of its protease and lipase systems (Larsen & Jensen, 1999; Le Dréan et al., 2010). However, uncontrolled growth of filamentous fungi during ripening of cheeses may cause undesirable effects that include off-flavours, anomalous textures, discolourations and accumulation of mycotoxins (Sengun, Yaman, & Gonul, 2008). For these reasons, there has been an increasing interest in analysing fungal populations associated with cheese over the last two decades (Erdogan, Gurses, & Sert, 2003; Kure & Skaar, 2000; Kure, Wasteson, Brendehaug, & Skaar, 2001; Lund, Filtenborg, & Frisvad, 1995; Montagna et al., 2004). More recently, important progress has been achieved with the use of molecular methods, which have improved the accuracy of identification, and have also allowed the determination of genetic variability and the description of new fungal populations and species (Flórez, Álvarez-Martín, López-Díaz, & Mayo, 2007; Hermet, Méheust, Mounier, Barbier, & Jany, 2012; Pannelli, Buffoni, Bonacina, & Feligini, 2012). However, it is generally considered that taxonomy of cheese fungi is still poorly understood (Ropars, Cruaud, Lacoste, & Dupont, 2012) and, more importantly, the information available about the ecology and physiology of these species is very scarce.

One of the most critical environmental parameters that determine the growth of fungi in any substrate is water availability. The concept of water activity (a_w) introduced by Scott (1957), has been widely used in food science literature to denote the total water content available for microorganisms, and is defined as the ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature. Water availability can be alternatively expressed as water potential (Ψ_w), which is the potential energy of water per unit volume relative to pure water in reference conditions, and it is measured in pressure units of megapascals (MPa) or bars (Magan, 2007). Ψ_w gives a good estimate of water balance, since it can differentiate between the forces required to remove water bound to the food matrix, the matrix potential (Ψ_m), and the forces due to colligative effects of dissolved compounds, the osmotic or solute potential (Ψ_s) (Jurado, Marín, Magan, & González-Jaén, 2008).

To control the quality of cheese, it is therefore important to understand the effects that changes in water status might have on growth kinetics of moulds. The objectives of this study were to isolate and identify fungi from a wide variety of hard and soft cheeses and to evaluate and compare their growth rates under the effect of ionic and non-ionic Ψ_s and Ψ_m.

2. Material and methods

2.1. Isolation of moulds from cheese

A total of 19 cheeses were purchased in local supermarkets in Spain during 2011 and 2012, stored at 4 °C, and analysed the day after collection. Samples included a wide variety of hard and soft cheeses made from cow, ewe, goat or mixed milk (Table 1). Ten small pieces from the surface layer of each cheese were excised under aseptic conditions, and directly plated onto 9-cm diameter Petri dishes containing 20 mL of oxytetracycline glucose chloramphenicol agar (Oxoid, Madrid, Spain). Plates were incubated aerobically in the darkness at 25 °C and observed daily. Colonies displaying different morphological characteristics were transferred to plates containing Sabouraud agar medium (Oxoid) to obtain monosporic cultures, and incubated at 25 °C for 7 days. Isolates were maintained at −20 °C as spore suspensions in 15% (v/v) glycerol.

2.2. DNA extraction and PCR amplification

Genomic DNA extractions of fungal isolates were undertaken using three mycelium discs excised from 5- to 7-day-old Sabouraud plate cultures, and making use of the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentrations were determined using a NanoDrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified by PCR using the primers and protocol described elsewhere (White, Burns, Lee, & Taylor, 1990). Amplification of the β-tubulin gene (*TUB2*) was performed using primers Bt2a and Bt2b according to Glass and Donaldson (1995). PCR-amplified fragments were purified using the UltraCleanTM PCR Clean-UpTM kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, and sent for direct sequencing to STABVIDA® (Caparica, Portugal). Automated sequencing of both strands was performed using the BigDye Terminator Kit by Applied Biosystems and the 96-capillary 3730xL DNA Analyzer by Applied Biosystems (Foster City, California, USA). The sequences were corrected using Chromas version 1.43 (Griffith University, Brisbane, Australia) and analysed and edited using Bioedit Sequence Alignment Editor version 7.0.9.0 (Hall, 1999). Sequences were deposited in the GenBank database, and numbers of accession are shown in Table 1.

2.3. Identification of fungi

Identification of fungi was based on the analysis of DNA sequences. Morphological and cultural observations were also done for isolates whose DNA sequences were uninformative. The keys developed by Frisvad and Samson (2004) were used for *Penicillium* isolates, whereas identification of isolates belonging to genus *Aspergillus* was done according to the keys developed by Hubka, Kolaric, Kubatova, and Peterson (2013).

2.4. Growth in relation to Ψ_s and Ψ_m

Sabouraud dextrose broth (Oxoid) supplemented with 2% agar (Oxoid) was used in this study. The Ψ_s was modified with the ionic solute sodium chloride or the non-ionic solute glycerol to −3.80, −7.72, −11.63 and −15.53 MPa corresponding to a_w of 0.975, 0.950, 0.925 and 0.900, respectively. These solutes were not added to the control medium (−0.7 MPa = 0.995 a_w).

The Ψ_m media were modified with polyethylene glycol 8000 (PEG 8000) (Sigma, Madrid, Spain) to obtain treatments of −3.80, −7.72, −11.63 and −15.53 MPa, respectively. PEG 8000 is known to act predominantly by matrix forces (Steuter, Mozafar, & Goodin, 1981). For matrically modified treatments, the agar was omitted and sterile circular discs of capillary matting (Nortene; 8.5 cm in diameter, 2 mm thick) were used to provide support for fungal growth. The capillary matting was overlaid with sterile cellophane sheets (28NP; Natureflex, Burgos, Spain). The control medium was similarly prepared, except that PEG 8000 was omitted (−0.7 MPa = 0.995 a_w).

2.5. Inoculation, incubation, and growth assessment

A 5-mm-diameter agar disk from the margin of a 7-day-old growing colony of each isolate grown at 20 °C was used to centrally inoculate each replicate and treatment. The plates were incubated at 20 °C for 10 days, and the experiment consisted of a fully replicated set of treatments with three replicates per treatment. Assessment of growth was made daily during the 10-day incubation period. For each colony, the mean radial mycelial growth was calculated by measuring two different colony radii in each of the three plates per combination of isolate and Ψ_m/Ψ_s treatment. The growth was corrected by subtracting the 5 mm diameter of the original plug of inoculum and plotted against time, and a linear regression was applied to obtain the growth rate as the slope of the line.

2.6. Statistical analysis of results

The linear regression of increase in radius against time (d) was used to obtain growth rates (mm d^{-1}) as indicated in 2.5 for each set of treatments. A two-way analysis of variance (ANOVA) of 'value of Ψ_w' (−0.7, −3.80, −7.72, −11.63 and −15.53 MPa) × 'type of Ψ_w' (NaCl, glycerol or PEG 8000) was carried out for each fungal strain. A one-way ANOVA was performed when interaction of both factors ('value of Ψ_w × type of Ψ_w') was significant. Subsequent post hoc analyses (Tukey's HSD tests of multiple comparisons) were carried out at a 95% confidence level.

3. Results

3.1. Identification of fungi

A total of 30 isolates were obtained from the 19 samples of cheese analysed, and their respective ITS sequences are shown in Table 1. The isolates belonged to 15 different species from genera *Penicillium* (16 isolates), *Geotrichum* (6 isolates), *Mucor* (4 isolates), *Aspergillus* (3 isolates), *Microascus* (1 isolate) and *Talaromyces* (1 isolate). Most of the isolates could be unambiguously identified by analysing exclusively the ITS sequence. However, morphological and cultural observations were necessary for identification of the species *Penicillium solitum*, *Penicillium discolor*, *Aspergillus glaucus* and *Aspergillus pseudoglaucus*, since they are known to carry identical ITS sequences to other species (Frisvad & Samson, 2004; Hubka et al., 2013). In addition, identification of isolate Zam2 (*Microascus manginii*) was done based on the analysis of *TUB2* sequence (number of accession KF298094), since no ITS amplification could be obtained with universal primers described by White et al. (1990). *TUB2* sequence was also obtained for isolate Qpe3 (number of accession KF298093), but this strain could not be

Table 1
Fungal species isolated from different varieties of cheese and respective accession numbers for their ITS (nuclear ribosomal internal transcribed spacer) sequences.

Cheese[a]	Country of production	Isolate	Species[b]	GenBank accession number (ITS)
Mahón[c;h]	Spain	Mah1	*Aspergillus varians**	KF298063
		Mah2	*Geotrichum candidum*	KF298068
Bettlematt[c;h]	Italy	Bet1	*Mucor racemosus**	KF298073
Bamalou[c;h]	France	Bam1	*Mucor circinelloides**	KF298072
Majorero[g;h]	Spain	Maj1	*Penicillium discolor**	KF298079
Ibores[g;h]	Spain	Mon1	*Penicillium roqueforti*	KF298083
		Mon2	*Penicillium solitum*	KF298084
		Mon3	*Mucor circinelloides*	KF298075
Zamorano[e;h]	Spain	Zam1	*Penicillium solitum**	KF298091
		Zam2	*Microascus manginii**	
		Zam3	*Penicillium chermesinum**	KF298090
Castellano[e;h]	Spain	Qpe1	*Penicillium chrysogenum**	KF298086
		Qpe2	*Penicillium discolor*	KF298087
		Qpe3	*Penicillium* spp.*	KF298088
Castellano[e;h]	Spain	Bal1	*Penicillium solitum*	KF298077
Manchego[e;h]	Spain	Man1-1	*Penicillium discolor*	KF298080
		Man1-2	*Talaromyces amestolkiae**	KF298092
		Man1-3	*Penicillium roqueforti**	KF298081
Manchego[e;h]	Spain	Man2-1	*Penicillium solitum*	KF298082
Manchego[e;h]	Spain	Man3-1	*Aspergillus glaucus**	KF298065
		Man3-2	*Aspergillus pseudoglaucus**	KF298064
Oropesa[e;h]	Spain	Oro1	*Penicillium discolor*	KF298085
Tomette des Alpes[c,g;h]	France	Tom1	*Geotrichum candidum*	KF298071
Gamoneu[c,g,e;h]	Spain	Gam1	*Penicillium olsonii**	KF298078
Thurgauer weinkäse[c;s]	Switzerland	Thu1	*Geotrichum candidum*	KF298070
Arzúa-Ulloa[c;s]	Spain	Arz1	*Penicillium discolor*	KF298076
Fleur du maquis[e;s]	France	Fle1	*Geotrichum candidum*	KF298066
		Fle2	*Mucor racemosus*	KF298074
Gour noir[g;s]	France	Gou1	*Geotrichum candidum*	KF298067
Quesuco de Liébana[c,e,g;s]	Spain	Que1	*Geotrichum candidum**	KF298069

[a] Superscript letters indicate: c, cow milk cheese; g, goat milk cheese; e, ewe milk cheese; h, hard cheese; s, soft cheese.
[b] An asterisk indicates isolates used for evaluating growth in relation to solute potential (Ψ_s) and matric potential (Ψ_m).

ascribed to any known species on the basis of the analysis of DNA sequences (ITS or *TUB2*), nor by morphological or cultural studies.

3.2. Fungal growth in relation to Ψ_s and Ψ_m

The results of the two-way ANOVA performed separately per each fungal strain showed significant interaction between 'value of Ψ_w' and 'type of Ψ_w' for all the strains tested (data not shown). Subsequently, one-way ANOVA and their correspondent Tukey's HSD tests were carried out to determine which values were statistically significant (Table 2).

Except for *M. manginii*, fungal growth was influenced by whether colonies were grown directly on the agar medium (Ψ_s) or on cellophane overlays (Ψ_m). This was evidenced by the lower growth obtained for most of the isolates at −0.7 MPa (control) in the matrically modified media (Ψ_m) (Fig. 1), with the only exception of *Aspergillus varians*, that was able to grow faster on the cellophane-overlaid medium. Therefore, absolute values of growth rate could not be compared between Ψ_s and Ψ_m data sets. In any case, fungal growth profiles were very different depending on the type of Ψ_w considered. The notable influence of Ψ_s, which produced clear profiles for optimal, permissive and marginal

Table 2
Results of Tukey's HSD tests performed separately per fungal strain for growth rate (mm d^{-1}) and carried out for value of water potential (Ψ_w) and type of Ψ_w.[a]

Species and isolate	Value of Ψ_w (NaCl/Glycerol/PEG 8000)					Type of Ψ_w (−0.70/−3.80/−7.72/−11.63/−11.53 MPa)		
	−0.7	−3.80	−7.72	−11.63	−15.53	NaCl	Glycerol	PEG 8000
M. circinelloides Bam1	aab	bac	bac	a*a	a*a	abccc	abc**	bccab
M. racemosus Bet1	aab	bac	cab	**a	**a	abc**	bac**	aaaab
P. roqueforti Man1-3	aab	bac	bac	a*b	**a	abcd*	bac**	abccb
P. chrysogenum Qpe1	aab	abc	abc	acb	b*a	cbacd	aabc*	bbaaa
P. discolor Maj1	aab	abc	aab	acb	b*a	bacde	aabc*	aaaba
P. solitum Zam1	aab	bac	aab	acb	b*a	cabcd	cabd*	aaaba
P. olsonii Gam1	aab	abc	bac	acb	b*a	babbc	bbac*	bbcab
P. chermesinum Zam3	aab	abc	bac	acb	b*a	aabcd	abac*	aabaa
Penicillium spp. Qpe3	aab	bac	aab	acb	b*a	cabde	cabd*	babdc
T. amestolkiae Man1-2	aab	cab	cba	**a	**a	abc**	abc**	babbc
M. manginii Zam2	aaa	abb	aba	b*a	b*a	cabcd	bab**	cbaab
A. glaucus Man3-1	aab	abc	aab	**a	**a	bab**	aaa**	babab
A. pseudoglaucus Man3-2	aab	bab	cab	cba	**a	baab*	dbac*	edcba
A. varians Mah1	bba	bac	cab	bca	b*a	abdce	abcd*	abbaa
G. candidum Que1	aab	bac	**a	**a	**a	ab***	ab***	aabcd

[a] Significant differences ($P < 0.05$) are indicated by different letters, where the ranking of letters (a > b > c > d > e) corresponds to the ranking of means. An asterisk indicates that no growth was detected.

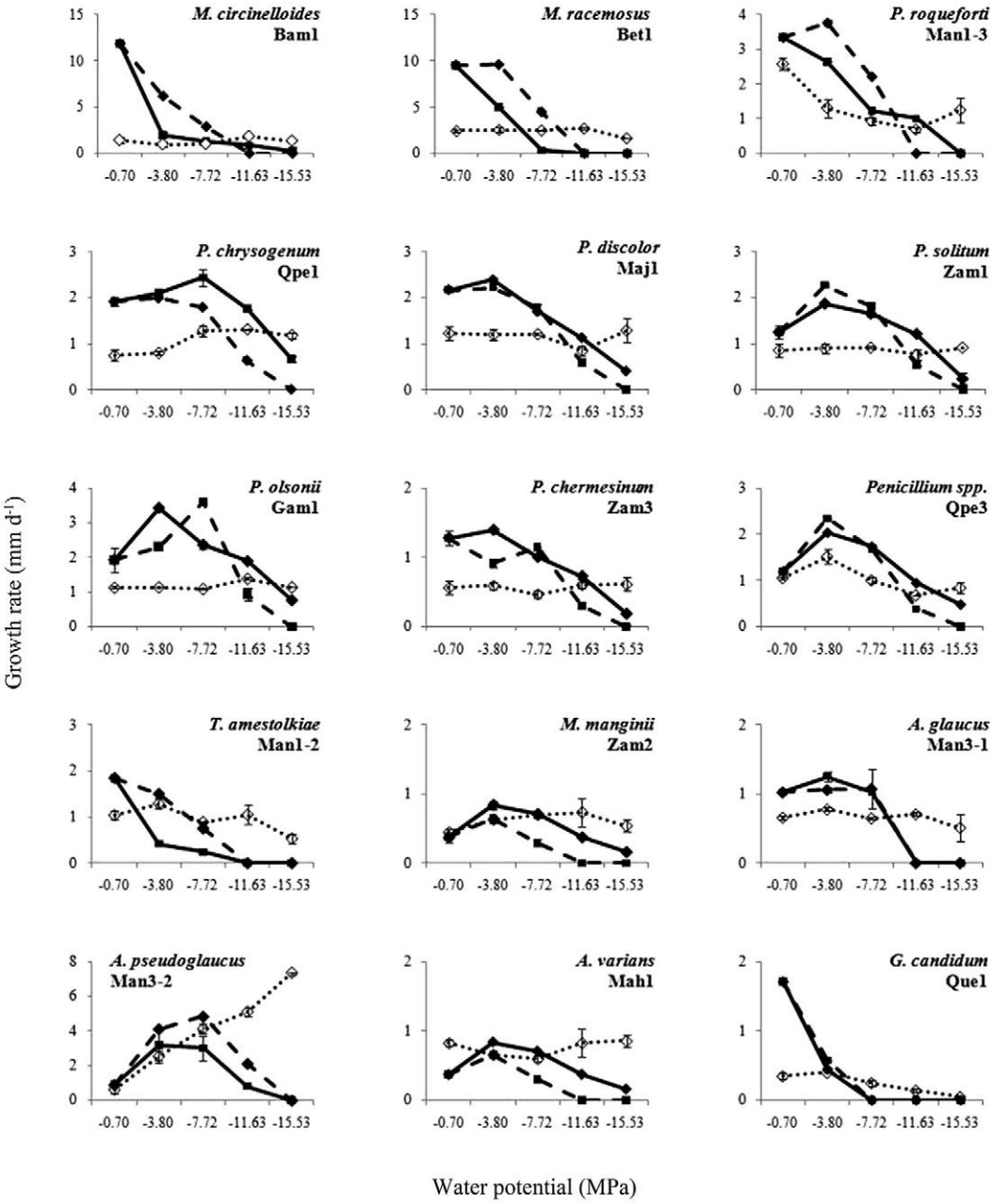

Fig. 1. Comparison of growth rates of fungal isolates in response to ionic (NaCl, ◆) and non-ionic (glycerol, ■) solute potential (Ψ_s) and matrix potential (Ψ_m) (PEG 8000, ◇) at 20 °C.

conditions for growth, contrasted with the profiles of Ψ_m. In this case, a more sustained growth was obtained for all the species, with the remarkable exception of *A. pseudoglaucus*, whose radial growth increased proportionally to the level of water stress imposed. It is noteworthy that all the strains were able to grow on the matrically modified medium at −15.53 MPa of Ψ_m and, moreover, a slight but statistically significant increase in growth rate, compared with control, was achieved at −15.53 MPa for the species *P. roqueforti*, *P. discolor*, *P. solitum* and *Penicillium* spp. Qpe3, and for *Penicillium chrysogenum* in the range of −7.72 to −15.53 MPa.

Geotrichum candidum was the most sensitive species to osmotic water stress, being its growth indistinctively restricted by both salt and glycerol, and it was the only isolate whose growth was completely inhibited by ionic and non-ionic Ψ_s at −7.72 MPa. Conversely, growth profiles of the rest of the species were differentially influenced by salt and glycerol. *Mucor racemosus*, *Mucor circinelloides*, *Talaromyces amestolkiae* and *A. pseudoglaucus* were clearly more tolerant to non-ionic stress whereas *P. chrysogenum*, *M. manginii*, and *A. varians* grew faster under ionic stress. The rest of the isolates presented more tolerance to salt or glycerol depending on the range of Ψ_w considered. This was particularly evident in the case of *Penicillium olsonii*, whose maximum growth rate was achieved at different Ψ_w depending on the solute considered: −3.8 MPa with salt (ionic), and −7.72 MPa with glycerol (non-ionic).

4. Discussion

Numerous papers have dealt with identification of fungal species associated with cheese, but less is known about how

environmental factors can affect their growth. In this work, the mycobiota associated with 19 different varieties of cheese is reported, and information about their growth profiles under different types of water stress is provided. Eight of the 15 species identified in this study belonged to species that have been frequently reported in cheese (Boutrou & Gueguen, 2005; Hermet et al., 2012; Ropars et al., 2012). This indicates, in terms of ecology, that these species can be considered as well adapted to this food. However, 7 of the 15 species are to our knowledge reported here for the first time in cheese (*Penicillium chermesinum*, *P. olsonii*, *A. varians*, *A. glaucus*, *A. pseudoglaucus*, *M. manginii* and *T. amestolkiae*). It must be noted though, that identifications were based on very recent taxonomical keys and molecular data, which have resulted in splitting of some species into new ones, and the update of the name of some species. This could explain, at least partially, the presence of previously unreported species. Nevertheless, we think that the wide variety of cheeses considered in this study, most of them never explored before, could also have influenced these findings.

Penicillium was clearly the dominant genus, especially on hard cheeses. Two strains belonging to section *Brevicompacta* were isolated. This is a taxon comprising a number of species that have been, until recently, poorly recognised (Pitt & Hocking, 2009). In fact, new species have been described within this section in the last few years (Frisvad, Houbrake, Popma, & Samson, 2013; Peterson, 2004; Serra & Peterson, 2007). One of these two isolates was identified as *P. olsonii*, a species associated with cured meats (López-Díaz, González, Moreno, & Otero, 2002), but apparently uncommon in cheese. The other isolate (Qpe3) could not be identified, but a phylogenetic tree built on the basis of both ITS and *TUB2* sequences that included all the known members from section *Brevicompacta*, revealed that it was most closely related to *Penicillium astrolabium* (data not shown). This species is associated with grapes and was firstly described by Serra and Peterson (2007). It is possible to hypothesise that Qpe3 could belong to a new species, although it would be desirable to perform more thorough taxonomic studies when new strains are available in future. For the rest of previously unreported species found in this work there is, unfortunately, little information available about their presence in foods.

The study of the influence of Ψ_w was of interest in this research because the status of water in cheese is an extremely variable parameter, which is influenced by the stage of ripening and the variety of cheese considered (Gaucel, Guillemin, & Corrieu, 2012; Pajonk, Saurel, & Andrieu, 2003; Saurel, Pajonk, & Andrieu, 2004). For example, water contents might range from values as low as 30% in hard cheeses (e.g., Parmigiano) to 80% in soft cheeses (e.g., mozzarella). Besides, salt addition during manufacturing and accumulation of ionic and non-ionic low molecular compounds generated through proteolysis, have an important effect on decreasing the total water available for microorganisms growing on cheese (Duggan, Noronha, O'Riordan, & O'Sullivan, 2008). The range of Ψ_w considered (from −0.70 to −15.53 MPa) was selected according to the values typically found in cheese (Hickey, Guinee, Hou, & Wilkinson, 2013). The results showed that whereas the decrease in water availability had a most inhibitory effect on growth of *T. amestolkiae*, *M. circinelloides*, *M. racemosus* and *G. candidum*, the species from genera *Penicillium* and *Aspergillus* were more tolerant to water restriction. This indicates that environmental conditions would have a huge influence on the dynamics of fungal populations while growing on cheese, favouring the growth of some species over others depending on the stage of ripening or the intrinsic characteristics of cheese. Previous reports have in fact pointed out that some mould species seem to be more widespread on certain cheeses or under particular environmental conditions. For example, *G. candidum* is known to be associated predominantly with soft cheeses (Boutrou & Gueguen, 2005), as was observed in this work, and *Mucor* species are important spoilage agents under conditions of high humidity (Spinnler & Leclercq-Perlat, 2007). The results obtained in this work help to explain these previous observations, although there are probably other factors involved in the distribution of fungal species which should be investigated.

A major finding was the different effect caused by ionic and non-ionic water stress in some species. In cheeses, Ψ_s is affected by the salt added during manufacturing and the accumulation of ionic and non-ionic low molecular compounds generated through proteolysis. The concentration of these osmolytes may increase over time as a result of water loss during ripening. Salt-in-moisture usually ranges from 2 to 23.9 in the final product (Guinee & Fox, 2004), but it can be even higher on the surface of cheeses that are immersed in saturated brine aqueous solutions, since a gradient is established from the rind to the core (Pajonk et al., 2003). Besides, accumulation of low molecular compounds through proteolysis is also very variable, since it depends on the activity of endogenous enzymes present in milk, the residual activity of milk coagulants (rennet) and the enzymes secreted by microbial communities (Gaucel et al., 2012). It could be then expected that the more halotolerant species, such as *P. chrysogenum*, could colonise cheeses with high-salt contents, or during the first days after brining, where more susceptible competitors would be inhibited. On the contrary, other species such as *A. pseudoglaucus* would prefer cheeses with high rate of proteolysis over those with high concentration of salt. The study of the relation between the presence of certain species and particular environmental conditions on cheese opens a vast field for further investigation.

Despite of the importance of food structure in water availability, there is a lack of information about Ψ_m in cheeses, probably because direct analysis of the structure of water in food systems is difficult to achieve (Mathlouthi, 2001). Differences in levels of water bound to cheese matrix are attributable to a large number of technological variables of the cheese making process. For example, it is known that hard cheeses have a more compact structure that restricts the water evaporation process, whereas a more open structure can be found on soft cheeses and on cheeses made with pasteurised milk (Buffa, Guamis, Saldo, & Trujillo, 2003). Other factors, such as the application of high pressure treatments, seem to result on a stronger binding of water to the cheese matrix (Johnston & Darcy, 2000). Our results showed, on one hand, that matric stress had less influence on variations of growth than solute stress, and on the other hand, that all the species were able to grow at the most extreme conditions of water availability, and some of them were even stimulated. Mycelial extension of other fungi such as *Aspergillus flavus*, *Fusarium verticillioides*, and of a range of basidiomycetes have previously been found to be significantly more sensitive to Ψ_m than to Ψ_s (Jurado et al., 2008). In the case of *A. pseudoglaucus*, growth was highly stimulated under matric stress. This ability to grow better on a substrate where water is physically constrained has also been reported in the species *Eurotium repens* and *Eurotium herbariorum*, and it could be a strategy of certain xerophilic fungi to access water in new regions (Huang, Chapman, Wilson, & Hocking, 2009). It seems therefore clear that the use of Ψ_w as the sole predictor for microbial stability of cheese might not be valid.

The data obtained in this work could have implications in the rational management of additives used for development of cheese analogues. For example, the use of starch has been proposed to achieve a higher microbial stability in these products, since this polysaccharide can entrap water within the matrix (Duggan et al., 2008). However, our results suggest that this strategy might be ineffective for limiting fungal growth. A possible alternative to

explore would be the use of replacement salts (magnesium, calcium or potassium), that until now has been assayed to produce healthier formulae of cheese analogues with lower sodium contents (Grummer & Schoenfuss, 2009), which could provide an additional benefit for fungal control.

This work has identified and characterised different mould species associated with a wide range of varieties of cheese. The study of the response of these species to distinct types of water stress indicates that some manufacturing parameters on the cheese making process, such as salting, pressing or ripening, could have a determinant impact on the mycobiota of cheeses. Therefore, further research should be carried out to investigate the influence of processing variables and their interactions on the different types of Ψ_w. This information would permit to identify critical points of the production process and to develop new strategies that enable to modulate fungal growth and therefore, to improve the final quality of cheeses.

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